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Award Number: W81XWH-12-1-0247

TITLE: Cell Lineage Analysis of Mouse Prostate Carcinogenesis

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REPORT DATE: July 2014

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
July 2014	Annual	1Jul 2013 - 30 Jun 2014

4. TITLE AND SUBTITLE
Cell Lineage Analysis of Mouse Prostate Carcinogenesis

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-12-1-0247

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The University of Pittsburgh

Pittsburgh, PA 15260

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Defining the origin of prostate cancer cells is fundamentally important and will guide future research to focus on cells from which prostate cancer cells are derived. Prostate cancer is thought to be derived from luminal epithelial cells in the prostate, because a hallmark of prostate cancer is the loss of basal epithelial cells and prostate cancer cells exhibit a luminal epithelial cell phenotype including the expression of AR and PSA. However, the luminal origin of prostate cancer has been challenged by a number of recent publications. This project will determine whether prostate cancer cells are derived from luminal or basal epithelial cells in an EAF2-/-; PTEN+/- mouse model, and determine whether luminal-derived prostate cancer cells behave differently from basal-derived prostate cancer cells. In the last year of the funding period, we have generated 2 breeding pairs (consisting of 1 male and 1 female) for Specific Aim 1, and 6 breeding pairs for Specific Aim 2.

15. SUBJECT TERMS: nothing listed

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	7	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Introduction

Defining the origin of prostate cancer cells is fundamentally important and will guide future research to focus on cells from which prostate cancer cells are derived. Prostate cancer is thought to be derived from luminal epithelial cells in the prostate, because a hallmark of prostate cancer is the loss of basal epithelial cells and prostate cancer cells exhibit a luminal epithelial cell phenotype including the expression of AR and PSA [1]. The capability of luminal epithelial cells as origin of prostate cancer is also supported by over expression of oncogenes such as cMYC and T-antigen or knockout of important tumor suppressor such as PTEN, specifically in prostate luminal epithelial cells. However, the luminal origin of prostate cancer has been challenged by a number of recent publications [2, 3]. This project will determine whether prostate cancer cells are derived from luminal or basal epithelial cells in an EAF2-/-; PTEN+/- mouse model, and determine whether luminal-derived prostate cancer cells behave differently from basal-derived prostate cancer cells.

Body

Genetic lineage tracing [4, 5] [6] and the EAF2-/-;PTEN+/- mouse prostate cancer model [7] will be used to determine whether prostate cancer cells are derived from basal and/or luminal epithelial cells in the prostate *in vivo*. All mice in this study will be on a C57BL/6J background.

Task 1: To determine whether prostate cancer can be derived from luminal epithelial cells in the EAF2-/-;PTEN+/- mouse prostate cancer model using PSA-CreER^{T2}-based genetic lineage tracing (months 1-30)

A. Obtain IACUC approval and generate PSA-CreER^{T2}; R26RmT/mG; EAF2-/-; PTEN+/- mice (month 1-16)

Status: IACUC protocol 12020202 was approved in 2013 during the previous status report period (2013). In the funding year 2013, we generated breeding pairs consisting of: (a) 1 female PSA-CreER^{T2}; EAF2-/-;PTEN+/-;R26RmT/mG+/- and 1 male EAF2-/-;PTEN+/-; R26RmT/mG+/-; (b) 1 female EAF2-/-;PTEN+/-; R26RmT/mG+/- and 1 male PSA-CreER^{T2}; EAF2-/-;PTEN+/-;R26RmT/mG+/-. These mice are deceased due to tumor generation (not in the prostate). Generation of mice is still ongoing and our current breeding pairs are identified in Table 1. Increased death has been noted in several animals with the following genotypes: PSA-CreER^{T2}; EAF2-/-;PTEN+/- at 1-2 mos of age, and PSA-CreER^{T2}; EAF2+/-;PTEN+/- at 6-12 mos of age. This has significantly hampered generation of the final cohort of 80 male PSA-CreER^{T2}; R26RmT/mG;EAF2-/-;PTEN+/- mice required to complete Specific Aim 1. Expected revised timeline months 1-30.

Table 1. Specific Aim 1 breeding pairs 2014

ID	Sex	DOB	PSA-CreERT2	EAF2	PTEN	R26RmT/mG
25	F	1/8/14	+	-/-	+/-	+/-
746	M	10/14/13	-	+/-	+/-	+/-
20	F	11/26/13	-	-/-	+/-	+/-
1	M	10/14/13	+	-/-	+/-	+/-

A subgroup of 4 male R26RmT/mG mice generated through the breeding strategy was utilized to determine the rate of spontaneous labeling in the prostate following multiple cycles of regression and regrowth. This subgroup of 4 animals was castrated at 10 weeks of age and subjected to 4 rounds of regression and regrowth as additional experimental controls. In the absence of the PSA-CreER^{T2} transgene, no labeled epithelial cells were detected in any of the R26RmT/mG animals (Figure 1). These results are in agreement with previous description of the R26RmT/mG murine model [8].

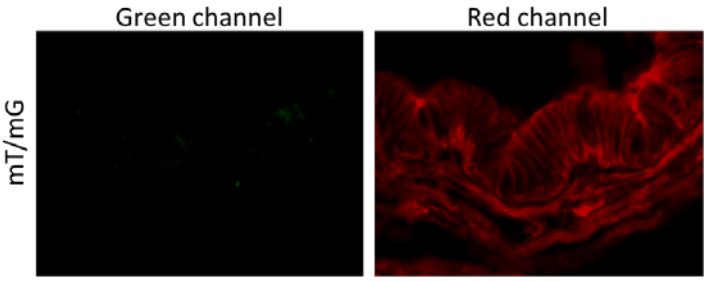

Figure 1. Images of fixed tissue sections of mT/mG male mice after 4 cycles of regression and testosterone-stimulated regrowth demonstrating minimal background fluorescence of mT and mG into the opposite channels.

B. Genotyping to verify genotype of genetically modified animals (month 1-30).

Status: Genotyping of pups is ongoing, tail clippings are taken for genotyping analyses at 21 days of age at weaning. Expected revised timeline months 1-30.

C. Intraperitoneal injection of tamoxifen to induce genetic combination to mark luminal epithelial cells (month 24-30)

Status: We are expecting the first experimental animals to be born within the next 90 days. These animals will be ready for intraperitoneal injection of tamoxifen at 6 weeks of age. Expected revised timeline months 24-30.

D. Histological and genetic lineage analysis of prostate cancer in mice (month 24-30)

Task 2: To determine whether prostate cancer cells can be derived from basal epithelial cells in the EAF2-/-;PTEN+/- mouse prostate cancer model (months 10-32).

A. Generate CK5-CreER^{T2}; R26RmT/mG; EAF2-/-; PTEN+/- mice (month 10-30)

Status: During funding year 2013, we encountered some difficulties with importing the CK5- or CK14-CreER^{T2}. The CK5-CreER^{T2} mice at Dr. Brigid Hogan's lab in Duke were positive with virus, and our animal facility was unable to accept the mice. Dr. Xin Li from Baylor had kindly offered us CK14-CreER^{T2} mice. Unfortunately, these animals were also infected with virus. Luckily, Jackson Lab has CK14-CreER^{T2} available. We placed order immediately and it took about 6 months for them to revive the colony and ship the animals to us. During the current funding year 2014 we have generated 6 intermediate breeding pairs with genotypes identified in Table 2 to produce mice with genotype CK5-CreER^{T2}; R26RmT/mG; EAF2-/-; PTEN+/-.

Table 2. Specific Aim 2 breeding pairs 2014

ID	Sex	DOB	CK5-CreERT2	EAF2	PTEN	R26RmT/mG
260	F	10/14/13	-	-/-	+/-	-
846	M	9/3/13	+	-/-	+/-	-
719	F	2/18/13	-	+/+	+/+	+/-
342	M	1/10/14	+	-/-	+/-	-
362	F	3/14/14	+	+/-	+/+	+/-
999	M	4/9/14	-	+/+	+/+	+/-
366	F	3/14/14	+	-/-	+/-	-
999	M	4/9/14	-	+/+	+/+	+/-
363	F	3/14/14	+	-/-	+/-	-
995	M	4/9/14	-	+/+	+/+	+/-
383	F	3/14/14	+	-/-	+/-	-
995	M	4/9/14	-	+/+	+/+	+/-

B. Genotype genetically modified animals (month 12-22).

Status: Genotyping of pups is ongoing, tail clippings are taken for genotyping analyses at 21 days of age at weaning.

C. Intraperitoneal injection of tamoxifen to induce genetic combination to mark luminal epithelial cells (month 14-20).

Status: We are expecting the first experimental animals to be born within the next 3 months. These animals will be ready for intraperitoneal injection of tamoxifen at 6 weeks of age. Expected revised timeline months 24-30.

D. Histological and genetic lineage analysis of prostate cancer in mice (month 16-32)

Task 3: To determine whether prostate cancer cells derived from luminal epithelial cells are different from those from basal cells in the EAF2-/-;PTEN+/- mouse prostate cancer model (months 16-36).

A. Immunohistochemical analysis of cell proliferation, luminal markers, basal markers, and neuroendocrine markers in luminal-derived prostate cancer (month 20-32).

B. Immunohistochemical analysis of cell proliferation, luminal markers, basal markers, and neuroendocrine markers in basal-derived prostate cancer (month 20-32).

C. Determine the effect of castration on luminal-derived and basal-derived prostate cancer (month 16-36).

D. Data analysis and manuscript writing (month 30-36).

Key Research Accomplishments

- Generated 2 breeding pairs (consisting of 1 male and 1 female) for generation of PSA-CreER^{T2}; EAF2-/-;PTEN+/-; R26RmT/mG+/- identified in Table 1.
- Determined that mG labeling does not spontaneously occur in mT/mG animals subjected to multiple cycles of prostate regression and testosterone-stimulated regrowth, see Figure 1.
- Generated 6 breeding pairs (consisting of 1 male and 1 female) for generation of CK5-CreER^{T2}; EAF2-/-;PTEN+/-; R26RmT/mG+/- identified in Table 2.

Reportable Outcomes

None.

Conclusions

None.

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